

Regeneration and Preliminary Characterization of Asian and African Okra (*Abelmoschus spp.*) Germplasm

Idi Saidou Sani,

University of Diffa, Faculty of Agronomic Sciences, Department of
Vegetable Production, UMR : Aridoculture and Oasis Crops, Diffa, Niger

Moustapha Grema,

University of Diffa, Faculty of Agronomic Sciences, Department of
Vegetable Production, UMR : Aridoculture and Oasis Crops, Diffa, Niger
Abdou Moumouni University of Niamey, Faculty of Technologic Sciences,
Department of Biology, Garba Mounkaila Laboratory, Niamey, Niger

Kumar Sanjeet,

AVRDC - The World Vegetable Center, AVRDC-ICRISAT Project,
Station de Sadoré/Niamey-Niger

Bakasso Yacoubou,

Abdou Moumouni University of Niamey, Faculty of Technologic Sciences,
Department of Biology, Garba Mounkaila Laboratory, Niamey, Niger

Ali Mahamane,

University of Diffa, Faculty of Agronomic Sciences, Department of
Vegetable Production, UMR : Aridoculture and Oasis Crops, Diffa, Niger
Abdou Moumouni University of Niamey, Faculty of Technologic Sciences,
Department of Biology, Garba Mounkaila Laboratory, Niamey, Niger

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Abstract

The morphological variability of 56 okra (*Abelmoschus spp.*) Accessions was evaluated over two years (2008 and 2009). Ten (10) agro-morphological characters were evaluated in the Sadoré station in Niger. The hierarchical ascendant classification (CAH) and the discriminant factor analysis (AFD) revealed four groups (GI, GII, GIII and GIV). The accessions in groups I and IV have high values of Lfm, Dfm, Height, and Nloc, Nepi and cons by low values. However, Flo or Fruct values are relatively higher in accessions of group III and IV than in accessions of group I. Similarly, accessions of group II have higher values in Flo and Fruct than group II. However, accessions of group II are much later in terms of which accessions in group I, which, in turn, have lower values in Flo and Fruct, so these are

compared to those of Group II. The results show that the most discriminating variables that make it possible to describe Variability among the groups identified are the diameter of mature fruits (Dfm), Plant height (Height) and branch of plant number (Nbrch). The characters analyzed can thus constitute basic criteria to differentiate accessions from other regions of West Africa and serve for a study of variability between the remains of okra accessions collected in Niger. Opportunities for improvement can also be considered to identify varieties that have a high adaptive potential due to climate change.

Keywords: *Abelmoschus spp.*, morphological variability, accessions, okra

Introduction

Okra (*Abelmoschus* spp.) is important vegetable crop in the developing and less developed countries of Asia and Africa. Among four known domesticated species of the genus *Abelmoschus*, *A. esculentus* is most widely cultivated for its edible pods in South and East Asia, West Central and East Africa, and the southern USA. The other cultivated species are *A. caillei* (edible leaves and pods) in humid forest zone of West Africa (Siemonsma, 1982), *A. manihot* (edible leaves) in Papua New Guinea and South Pacific Islands and *A. moschatus* (seeds for ambrette and animism practices) in India and South Togo and Benin (Hamon *et al.*, 1995). Okra has potential to significantly contribute to increased livelihoods of both rural and urban populations (Kumar *et al.*, 2011). This is especially because of its robust nature, dietary fibers and distinct seed protein balanced in both lysine and tryptophan amino acids (unlike the proteins of cereals and pulses) it provides (DS/MDA, 2008). Okra has also been predicted as an industrial crop (Camciuc *et al.*, 1998). However, until recently, no attention was paid to its improvement in the international research program in past (Duzyaman, 1997). During 2007, we initiated okra improvement activities and in this report results on preliminary morphological characterization of some germplasm accessions are being presented and discussed.

Material and methods

This study had been conducted at ICRISAT station of Sadoré (Situating in the south-west part of Niger at 45 km, south of Niamey) during two rainy seasons (2008 and 2009). The minimal and maximal temperature means that have been saved during these two seasons were respectively 23.2 °C and 35.5 °C for 2008 year; 22.8 °C and 35.18 °C for 2009 year. The rainfall was also respectively 480.6 mm and 515.6 mm for 2008 and 2009.

Fifty-six accessions of three species, namely, *A. esculentus*, *A. manihot* var. *tetraphyllus* and *A. manihot* var. *manihot* (re-assigned as *A. caillei* after Siemonsama, 1982) originated from 12 countries of Asia and Africa were

utilized (Table 1). These accessions were introduced from National Plant Germplasm System (NPGS), United States and AVRDC – The World Vegetable Center, Taiwan during 2007 and comprised of 48 locally collected genotypes, six improved open pollinated varieties and two hybrids (F₁). These accessions were part of about 250 germplasm accessions regenerated by AVRDC/ICRISAT (International Crops Research Institute for the Semi Arid Tropics) Project in Niger.

Table1: Origin of different okra lines

Origine	Varieties	Code_Variétés
Bangladesh	TOT-0546	1e
Bangladesh	TOT-4676	2e
India	GRIF 12789	3t
India	GRIF 12790	4t
India	Grif 13133	5e
India	Grif 13149	6e
India	Grif 13164	7e
India	Grif 13191	8e
India	Grif 13433	9e
India	Grif 13435	10e
India	PI 116453	11e
India	PI 116454	12e
India	PI 249620	13e
India	PI 370028	14e
India	PI 370029	15e
India	PI 548700	16e
India	RCA-1	17e
India	RAKSHAK F1	18e
Malaysia	TOT-2742	19e
Philippines	TOT-2788	20e
Philippines	TOT-3133	21e
Philippines	TOT-3134	22e
Philippines	TOT-3139	23e
Philippines	TOT-3142	24e
Philippines	TOT-3143	25e
Philippines	TOT-3442	26e
Taiwan	TOT-6000	27e
Taiwan	TOT-6018	28e

Origine	Varieties	Code_Variétés
Thailand	TOT-3854	29e
Thailand	TOT-3855	30e
Thailand	TOT-3851	31e
Thailand	TOT-3859	32e
Benin	PI 496944	33c
Benin	PI 496945	34c
Benin	PI 496452	35e
Benin	PI 496957	36e
Benin	PI 496954	37e
Benin	PI 496955	38e
Cameroon	PI 535944	39e
Ghana	PI 497026	40c
Ghana	PI 280063	41e
Ghana	PI 496620	42e
Ghana	PI 496616	43e
Ghana	PI 496617	44e
Ghana	PI 496618	45e
Ghana	PI 496619	46e
Guinea	PI 496640	47e
Guinea	PI 496634	48e
Guinea	PI 496631	49e
Guinea	PI 496635	50e
Guinea	PI 496639	51e
Niger	KONNI	52e
Niger	GAYA	53e
Niger	TERA	54e
	OKML-LIMA	
Senegal	F1	55e
Senegal	VOLTA	56e

The experiment was conducted according to a Fisher block device completely randomized with four repetitions. In each block, an accession is represented by twenty plants which are separated by a distance of 0.3 m between the poquet, and a distance of 0.5 m between the line. The lines of the

same block are separated by one meter from each other and successive blocks of two meters.

Twenty plants of each accession were raised during two rainy seasons (2008 and 2009) and observations were recorded on 10 morphological descriptors. Five plants in each accession were randomly selected to record observations on plant height (Height) and number of branch per plant (Nbrch). Three pods from each plant were used to determine length of immature (horticultural maturity) fruit (Ljm), diameter of immature fruits (Djf), length of mature fruit (Lfm), diameter of mature fruits (Dfm), number of locule (Nloc) and number of epicalyx (Nepi). Days to 50% flowering (Flo) and days to 50% fruiting (Fru) were determined on population basis. Using two season’s data, the average clustering method was used with mean of two seasons data on ten quantitative characters to prepare dendrogram (Fig. 1).

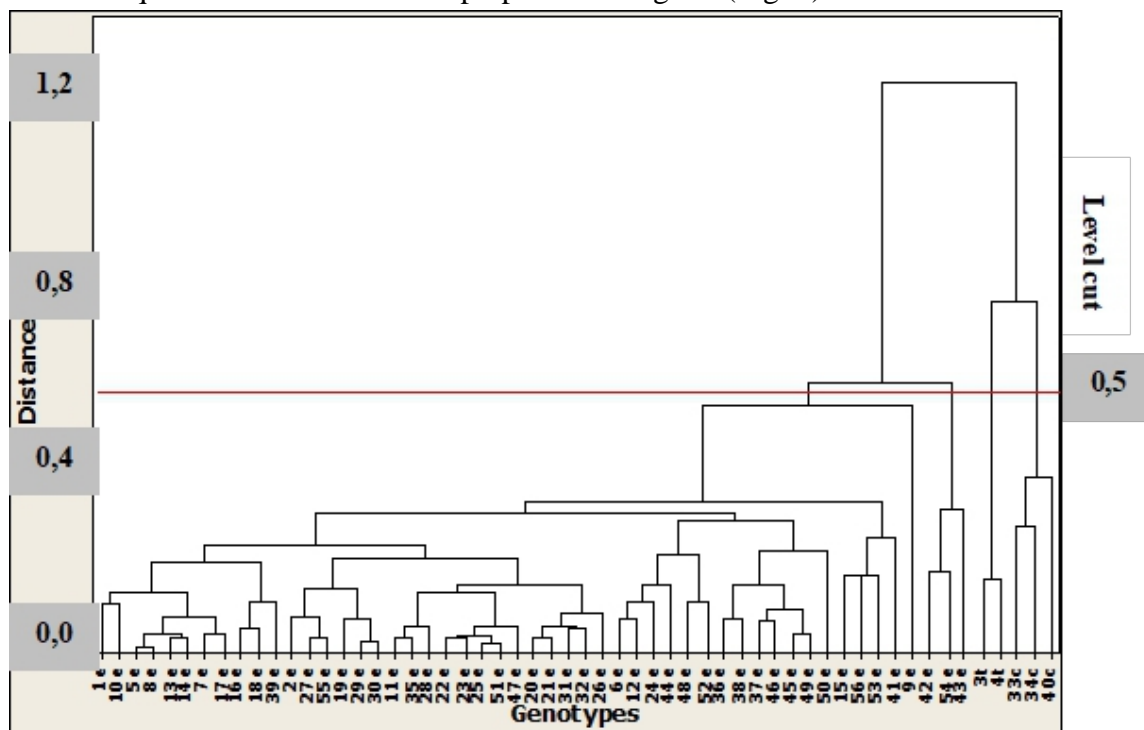


Fig.1: Dendrogram of 56 okra genotypes on the basis of genetic distance

The factorial analysis discriminant had been making to discriminat the different group obtained after HCA analysis. For all analysis data, two software were being used. The Minitab14 software for preparing dendrogram and XLSTAT-pro-V7.5.2 software for Factorial Analysis Discriminant (FAD).

Results

The dendrogram resulted from average linkage clustering of 10 quantitative data allowed a very clear-cut grouping of three *Abelmoschus* spp. (Fig 1). The first and second set of clusters included all 51 accessions of *A. esculentus*. The first cluster had 48 accessions collected from different countries of Asia and West and Central Africa (WCA), while the second cluster had only three accessions collected from West Africa, two from Ghana and one from Niger. The third cluster comprised of two accessions of *A. manihot* var. *tetraphyllus* from India and the fourth cluster had three *A. caillei* accessions collected from Benin and Ghana (Fig.1 & Table 1). Although dendrogram clearly separate accessions according to species, clustering of accessions accordingly to geographical origin and accession type (local landrace, improved cultivar and hybrids) could not be observed.

• Results of discriminant analysis on four groups already identified

The results of the FDA applied to data from four groups identified are first to feel the Mahalanobis distances (McLachlan, 1992) between the groups identified by the HCA and their significance (Table 2). The results in Table 2 indicate that there is a very highly significant difference 0.0001 between the 4 groups on the basis of all the variables considered. Otherwise, all the variables have contributed to discriminate the 4 groups. These results are confirmed by the multivariate test through statistical Wilks' Lambda (probability<0.004).

Table 2. Test results of discrimination by Mahalanobis distance

	Grp I	Grp II	Grp III	Grp IV
Grp I	0	-	-	-
Grp II	164.1***	0	-	-
Grp III	135.7***	123.3***	0	-
Grp IV	58.2***	237.8***	77.7***	0

*** Significant up to 0.05%

Moreover, examination of the values of the F statistic of Fisher and coefficient of determination R² indicated that the variables Dfm, Height and Nbrch are more discriminating with R² values relatively high compared with other variables (Table 2).

Table2. Results of univariate tests and multivariate discriminant power groups by the variables: R-square values and significance.

Variable	R ²	F(3)*	F Value
Lfm	0.338	33.978	< 0.0001
Ljm	0.238	34.879	< 0.0001
Dfm	0.514	16.372	< 0.0001
Djf	0.138	36.879	< 0.0001
Flo	0.192	72.938	< 0.0001
Fruct	0.160	91.068	< 0.0001
Height	0.631	10.118	< 0.0001
Nbrch	0.648	9.426	< 0.0001
Nloc	0.385	27.647	< 0.0001
Nepi	0.049	339.789	< 0.0001
Wilks' Lambda statistics		(ddl =52	<0.0001
(3)* : ddl = 3			

Height: Plant height; **Nbrch** : Number of branch per plant ; **Ljm** : length of immature (horticultural maturity); **Djf** : fruit diameter of immature fruits ; **Lfm** : length of mature fruit; **Dfm** : diameter of mature fruits ; **Nloc** : number of locule ; **Nepi** : number of epicalyx ; **Flo** : Days to 50% flowering ; **Fruct** : days to 50% fruiting

Moreover, examination of the eigenvalues of canonical axes and their significance (Table 3) showed only the first three canonical axes are significant (probability <0.0001) and concentrate all alone (100%) information contained in the original variables.

Table3. Proportions of information concentrated on the canonical axes, canonical correlation and significance of canonical axes.

Axes	Proportions	Cumulated proportion	Prob
1	82.269	82.269	<0.0001
2	16.543	98.812	<0.0001
3	1.188	100.000	<0.0001

The description of correlations between initial variables and the first discriminant axis (CAN1) revealed that only high values of Lfm, Dfm, Height, Nloc and Nepi are associated with axis 1. This trend is confirmed by the two discriminant axes CAN2 and CAN3. Indeed, in general, genotypes with high values in group Lfm, Dfm, Height, Nepi and Nloc are genotypes that have a high plant size, large number of bun and epicalyx locule but also a great mature fruit diameter (Table 4). Axes 1 and 2 already take into account the information contained in the axis 3. Thus, only these two axes will be used to describe the 4 groups.

Table 4. Correlations between initial variables and discriminant axes.

Variables	Can1	Can2	Can3
Lfm	0.729	-0.416	0.079

Ljm	0.133	0.277	0.125
Dfm	0.659	0.222	-0.288
Djf	0.323	0.477	0.113
FLO	0.358	0.896	0.069
Fruct	0.404	0.895	0.005
Height	0.587	0.192	0.124
Nbrch	0.233	0.377	0.782
Nloc	0.794	0.079	0.042
Nepi	0.959	-0.273	0.021

The projection of the 4 groups in the system of canonical axes 1 and 2 (Fig.2) indicates that the first canonical axis has a discriminatory power higher than the second canonical axis. Indeed, it may be noted that Group II is more distant from groups I and IV, closer to each other with respect to the axis 1, while groups III and IV are particularly discriminated against by the axis 2. Groups I and IV are also discriminated against in some measure by the axes 2.

Thus, we conclude that individuals in groups I and IV have high values of Lfm, Dfm, Height, and Nloc, Nepi and cons by low values when considering parameters such as Flo or Fruct unlike individuals in group II. However, Flo or Fruct values are relatively higher in individuals of group III and IV than in individuals of group I. Similarly, individuals of group I have higher values in Flo and Fruct than group II. However, individuals of group II are much later Fruct Flo and values that individuals in group I, which, in turn, have lower values in Flo and Fruct, so these genotypes are much earlier compared to those of Group II. We can also say without risk of error that genotypes groups III and IV are much later (with a mean value (Flo = 120.0 and Fruct = 130.0; Flo = 111.6 and Fruct = 128.6 respectively for group III and IV) on phenological traits of both varieties of okra species, compared to a group of genotypes that are much earlier (with value Average for genotypes of the said group are 50.575 and 58.775 respectively for Flo and The Fruct) Table 5.

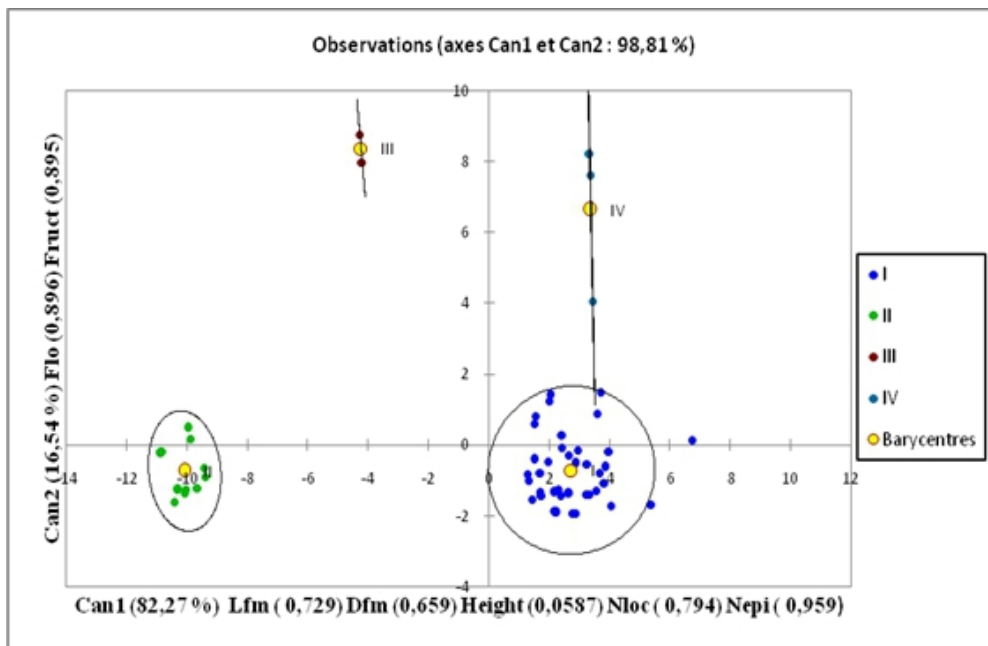


Fig.2: Projection of four groups in the plane formed by the first two canonical axes.

Table 5. Characteristics of the groups: mean values and standard deviations of the parameters by group, value of the Newmann Keuls statistic and their significance.

Variables	Grp I		Grp II		Grp III		Grp IV		Global		Test statistics	
	m	s	m	s	m	s	m	s	m	s	F	Prob
Lfm	14.938 ^a	2.24	7.277 ^b	1.234	5.025 ^c	1.63	9.55 ^b	2.9	9.1975 ^b	4.250	162.990	0.055
Ljm	14.932 ^a	1.03	7.259 ^c	2.522	5.02 ^d	1.13	8.989 ^b	3.12	9.05 ^b	4.245	5446.647	0.090
Dfm	1.668 ^b	0.12	0.595 ^a	0.18	1.24 ^c	0.21	2.527 ^d	0.94	1.5075 ^b	0.810	0.211	0.009
Djf	1.65 ^a	0.38	0.581 ^a	0.23	1.22 ^a	0.23	2.52 ^a	1.32	1.49275 ^a	0.814	0.206	0.842
FLO	50.575 ^a	6.78	29 ^a	2.45	120 ^a	15.25	111.667 ^a	9.89	77.8105 ^a	44.909	1.479	0.503
Fruct	58.775 ^a	3.25	32.636 ^a	4.25	130 ^a	9.1	128.667 ^a	10.13	87.5195 ^a	49.451	1.125	0.555
Height	57.715 ^a	5.01	25.818 ^b	4.7	60.95 ^c	7.28	66.9 ^d	5.78	52.84575 ^a	18.416	1.432	0.008
Nbrch	4.97 ^a	2.02	3.136 ^d	1.73	7.85 ^b	2.34	4 ^c	1.38	4.81525 ^a	2.081	6.827	<0.0001
Nloc	6.118 ^a	1.43	2.955 ^a	0.28	5 ^a	1.44	6.533 ^a	2.06	5.1515 ^a	1.601	1.011	0.575
Nepi	10.3 ^a	2.34	4.864 ^a	1.02	5.15 ^a	2.19	8.633 ^a	2.57	7.23675 ^a	2.666	3.601	0.349

Height: Plant height; **Nbrch** : Number of branch per plant ; **Ljm** : length of immature (horticultural maturity); **Djf** : Diameter of immature fruits ; **Lfm** : length of mature fruit; **Dfm** : diameter of mature fruits ; **Nloc** : number of locule ; **Nepi** : number of epicalyx ; **Flo** : Days to 50% flowering ; **Fruct** : days to 50% fruiting . On each line the values accompanied by different letters are significantly different at the probability threshold ($\alpha = 0.05$).

Discussion

The results of this work allowed us to highlight the existence of the great diversity of okra genetic resources in West Africa but also in other continents of the world. The variability observed within the varieties indicates that there are at least groups which are different from each other on the basis of the quantitative characters thus studied. It also allowed us to understand that

even if the varieties are geographically distant some of them have morphological and genetic characters that are close to each other, which explains the different homogeneous groups obtained from the factorial discriminant analysis (AFD) and hierarchical ascending classification (CAH). However, cultivated varieties, even if they are genetically not very variable, have high potential for ecotypic differentiation due to abiotic and biotic stress (case of attack of nematodes and whitefly), which would need to be better exploited internationally (Sani, 2008). The study of wild relatives, in terms of phylogeny or possibilities of introgression, still little developed, also gives hope for further genetic progress.

Multivariate analyzes across the CAH and AFD showed that the variability of our accessions is structured in four groups on the basis of diameter of mature fruits (Dfm), Plant height (Height) and number of branch per plant (Nbrch) characters. It emerges from the high representativity of the first two canonical axes of the AFD (98.81%), the existence of a strong genotypic and phenotypic organization of the studied material. The morphological and phenological dissimilarities observed between the different phenotypic groups suggest that accessions are maintained under very different evolutionary processes in their respective agroecosystems. Agroecosystems are likely to exert very selective pressure Genotype variables (Sadiki and Jarvis, 2005) as well as anthropogenic pressures (Robert et al., 2004). In fact, farmers' management of seeds such as selective sorting, post-harvest techniques and agricultural practices leads to selection leading to the maintenance or even the creation of significant phenotypic diversity (Robert et al. ., 2005).

These evolutionary factors could be the causes of the clear agromorphological and phenological differentiation observed between accessions. This constant evolution, influenced by the pedological, climatic and anthropic factors in the different agroecosystems, gives to the accessions characteristics of their own (Azam-Ali et al., 2001).

This type of observation, which shows the high intra-accession and inter-accession variability, has been described by several authors in different species cultivated in West Africa (Rouamba et al., 2001, Demol et al., 2002, Barnaud et al. al., 2007, Bakasso 2010, Antoine et al., 2012, Idi-saidou et al., 2014).

Okra (*Abelmoschus* spp. *esculentus*) and African okra (*A. caillei*) are well recognized vegetable crops of African and Asian continents. Although *A. caillei* cultivated in West and Central Africa (WCA) and account only 5% of total world okra production, it is an important indigenous crop of humid and tropics (Siemonsma 1982a, b). Based on detail chromosome and crossing analysis African okra (*A. caillei* syn. Guineen type) was proposed to be an amphipolyploid derived from *A. esculentus* (common okra) and *A. manihot*

(Siemonsama, 1982a ; Siemonsama, 1982b) and a hypothesis put forward that African okra is gradually replacing the common okra in the humid region of West Central Africa (Siemonsama 1882a). On the basis of further study on geographical distribution of *A. esculentus* and *A. caillei* showed that both are cultivated in most of the villages strat form Gulf of Guina to southern limit of Sahel, however, genetic integrity of these two species is not found to be threatned due to lack of spontaneous natural crossing between these two species (Hamon and Hamon 1992).

Conclusion

The variability observed within the accessions studied is an excellent argument for the conservation of traditional okra cultivars in the different areas of their production. This significant variability within the species could be favored its mode of reproduction. The place of local accessions in the plant resources of cultivated plants being no longer to be demonstrated, special attention must be paid to the regions that ensure the sustainability of this source of variability. The characters analyzed can thus constitute basic criteria for differentiating accessions from other producing regions of the species in Niger and can be used in morphological diversity studies of okra in West Africa and Asia. These results show the need to test the genetic approach by using molecular markers to better understand the global genetic diversity. This diversity compared with that identified by the perception of the farmers, but also to the diversity obtained after the phenotypic evaluation of the collected accessions allows a rational use of this resource in the programs of the improvement of the okra of Niger and those elsewhere.

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